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Safety and pharmacokinetics of anti-TFPI antibody (concizumab) in healthy volunteers and patients with hemophilia: a randomized first human dose trial

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Abstract: **BACKGROUND:** Prophylaxis with either intravenous (i.v.) factor VIII (FVIII) or FIX is the gold standard of care for patients with severe hemophilia. A monoclonal antibody (concizumab) targeting tissue factor pathway inhibitor (TFPI) that can be administered subcutaneously (s.c.) has the potential to alter current concepts of prophylaxis in hemophilia. **OBJECTIVES:** To evaluate the safety and describe the pharmacokinetics and pharmacodynamics of single-dose concizumab in healthy volunteers and patients with hemophilia A or B. **METHODS:** In this first human dose, phase 1, multicenter, randomized, double-blind, placebo-controlled trial escalating single i.v. (0.5-9000 g kg⁻¹) or s.c. (50-3000 g kg⁻¹) doses of concizumab were administered to healthy volunteers (n = 28) and hemophilia patients (n = 24). **RESULTS:** Concizumab had a favorable safety profile after single i.v. or s.c. administration. There were no serious adverse events and no anti-concizumab antibodies. No clinically relevant changes in platelets, prothrombin time, activated partial thromboplastin time, fibrinogen, or antithrombin were found. A dose-dependent procoagulant effect of concizumab was seen as increased levels of D-dimers and prothrombin fragment 1 + 2. Nonlinear pharmacokinetics of concizumab was observed due to target-mediated clearance. A maximum mean AUC_{0-∞} of 33 960 h g mL⁻¹ and a maximum mean concentration of 247 g mL⁻¹ was measured at the highest dose. **CONCLUSIONS:** Concizumab showed a favorable safety profile after i.v. or s.c. administration and nonlinear pharmacokinetics was observed due to target-mediated clearance. A concentration-dependent procoagulant effect of concizumab was observed, supporting further study into the potential use of s.c. concizumab for hemophilia treatment.

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ORIGINAL ARTICLE

Safety and pharmacokinetics of anti-TFPI antibody (concizumab) in healthy volunteers and patients with hemophilia: a randomized first human dose trial

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Summary. *Background:* Prophylaxis with either intravenous (i.v.) factor VIII (FVIII) or FIX is the gold standard of care for patients with severe hemophilia. A monoclonal antibody (concizumab) targeting tissue factor pathway inhibitor (TFPI) that can be administered subcutaneously (s.c.) has the potential to alter current concepts of prophylaxis in hemophilia. *Objectives:* To evaluate the safety and describe the pharmacokinetics and pharmacodynamics of single-dose concizumab in healthy volunteers and patients with hemophilia A or B. *Methods:* In this first human dose, phase 1, multicenter, randomized, double-blind, placebo-controlled trial escalating single i.v. (0.5–9000 $\mu\text{g kg}^{-1}$) or s.c. (50–3000 $\mu\text{g kg}^{-1}$) doses of concizumab were administered to healthy volunteers ($n = 28$) and hemophilia patients ($n = 24$). *Results:* Concizumab had a favorable safety profile after single i.v. or s.c. administration. There were no serious adverse events and no anti-concizumab antibodies. No clinically relevant changes in platelets, pro-

thrombin time, activated partial thromboplastin time, fibrinogen, or antithrombin were found. A dose-dependent procoagulant effect of concizumab was seen as increased levels of D-dimers and prothrombin fragment 1 + 2. Non-linear pharmacokinetics of concizumab was observed due to target-mediated clearance. A maximum mean $\text{AUC}_{0-\infty}$ of 33 960 $\text{h } \mu\text{g mL}^{-1}$ and a maximum mean concentration of 247 $\mu\text{g mL}^{-1}$ was measured at the highest dose. *Conclusions:* Concizumab showed a favorable safety profile after i.v. or s.c. administration and nonlinear pharmacokinetics was observed due to target-mediated clearance. A concentration-dependent procoagulant effect of concizumab was observed, supporting further study into the potential use of s.c. concizumab for hemophilia treatment.

Keywords: hemophilia; mAb 2021; pharmacokinetics; safety; tissue factor pathway inhibitor.

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Introduction

Severe hemophilia A and B are inherited bleeding disorders characterized by recurrent and spontaneous bleeding into joints, muscles, and soft tissues, resulting in musculoskeletal damage and chronic disability. Historically, without treatment, fatal bleeding was common in patients with severe hemophilia [1], but the availability of high-purity clotting factor concentrates has clearly improved their management [2]. In particular, primary

prophylaxis with regular venous infusion of the deficient clotting factor is recommended [3] to avoid the risk of life- and limb-threatening bleeds. Secondary prophylaxis, started later in life after multiple joint bleeds, and even after the onset of joint damage, may also be beneficial [4].

Despite the World Health Organization, among others, supporting prophylaxis as the standard of care, many patients with severe hemophilia still receive on-demand treatment for bleeding episodes. One barrier to prophylactic treatment is the requirement for venous access. This can be problematic, especially in small children, and central venous access devices are associated with infections and thrombotic occlusions [5]. Another potential hindrance is the relatively short half-life ($t_{1/2}$) of available coagulation factor VIII (FVIII) and FIX products, requiring patients to self-infuse between twice a week and up to every second day, and having a negative effect on compliance and quality of life [6,7]. Advances in protein engineering have facilitated the development of FVIII and FIX with long $t_{1/2}$ [8–11]. While this approach has been successful for FIX [12–15], similar gains have not been seen for FVIII, where $t_{1/2}$ appears to be more dependent on von Willebrand factor levels [16].

There is considerable interest in alternate targets and modes of drug administration, especially for subcutaneously administered agents with the potential for improved compliance. One potential target is tissue factor pathway inhibitor (TFPI), a multivalent, Kunitz-type proteinase inhibitor (KPI) with three KPI domains (Fig. S1) [17,18]. TFPI is a potent inhibitor of the initiation pathway, in which inhibition of the tissue factor-activated FVII (TF-FVIIa) complex is dependent on FXa inhibition. TFPI circulates in three isoforms, and the microvascular endothelium is believed to be the major source of TFPI, where it is associated with the cell surface after secretion. Following injury, the TF-FVIIa complex activates FIX to FIXa and FX to FXa—this FXa generation is tightly regulated by TFPI. The TFPI KPI-2 domain inhibits the active site of FXa, enhanced by protein S. Inhibition of the TF-FVIIa complex by TFPI requires initial binding of KPI-2 to FXa, and subsequent formation of a TF-FVIIa-FXa-TFPI complex, in which the KPI-1 domain inhibits FVIIa (Fig. S1). Novo Nordisk has developed a humanized monoclonal antibody against TFPI, concizumab (mAb 2021), with high affinity for the KPI-2 domain of TFPI, the binding site of FXa (Fig. S2). By preventing FXa binding to TFPI, concizumab also prevents TFPI inhibition of TF-FVIIa complex, thereby abrogating TFPI function as a regulator of the TF pathway, resulting in enhanced FXa and thrombin generation *in vitro*. Nonclinical studies characterizing the effect of concizumab *in vitro* and *in vivo* [19] showed that concizumab binds with high affinity to both free and cell surface-bound TFPI (a substantial fraction of TFPI is associated with endothelial cell surfaces). In hemophilia

blood and plasma, improved clot formation under hemophilia-like conditions was noted in a concentration-dependent manner [19]. In a rabbit hemophilia bleeding model, an intravenous (i.v.) or subcutaneous (s.c.) concizumab dose substantially reduced cuticle bleeding, with an effect comparable to that of recombinant FVIIa [19]. The efficacy data in rabbits demonstrate the potential effect of concizumab in efficiently preventing bleeding in hemophilia patients.

Monoclonal antibodies bring the advantage of a long $t_{1/2}$ and the possibility of s.c. administration. Thus, concizumab may constitute a novel approach for the management of hemophilia patients with and without inhibitors and would present the first s.c. prophylactic option. This report describes the first-in-human clinical trial (explorerTM1) to investigate the safety and to describe the pharmacokinetics (PKs) and pharmacodynamics (PDs) of single-dose concizumab administered i.v. or s.c. in healthy volunteers and in patients with hemophilia A or B.

Methods

Trial design

ExplorerTM1 was a first human dose, phase 1, multicenter, randomized, double-blind, placebo-controlled, single-dose, dose-escalation trial. This multinational, multiethnic trial enrolled participants at 13 sites in nine countries (Austria, Denmark, Germany, Malaysia, South Africa, Spain, Switzerland, Thailand, and the United Kingdom). ExplorerTM1 (clinicaltrials.gov identifier: NCT01228669) was approved by local ethical committees, independent ethics committees, or institutional review boards and was conducted in accordance with the Declaration of Helsinki and ICH Good Clinical Practice. Written informed consent was obtained from all participants prior to trial-related activities.

Escalating single i.v. or s.c. doses of the trial product were administered to healthy volunteers and patients with hemophilia A or B according to the dosing schedule in Fig. 1. Trial participants were assigned to the lowest randomization number using a computer-generated randomization list provided by Clinical Supplies Coordination, Novo Nordisk A/S. Within each dose cohort, trial participants were randomized 3:1 to receive a single dose of concizumab ($n = 3$) or placebo ($n = 1$). Due to the protein content, the appearance of reconstituted concizumab and placebo/diluent differed. Thus, a qualified, unblinded person prepared the dosing syringes, after which no differences in the appearance of the two solutions could be seen. Investigators and trial participants were blinded to treatment.

Dosing was initiated in healthy volunteers and, at predefined switching criteria, was continued in hemophilia patients. Switching criteria were defined as a consistent

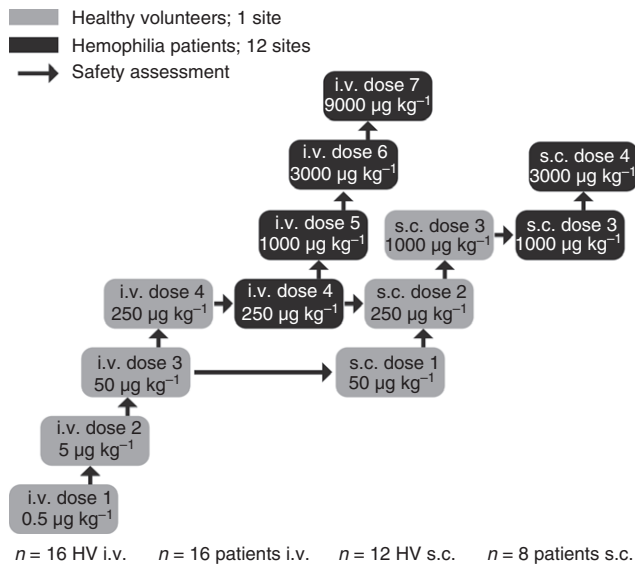


Fig. 1. Explorer™1 dosing schedule. Four healthy volunteers (HV) or hemophilia patients were randomized in each cohort (concizumab, *n* = 3; placebo, *n* = 1). Each participant received one dose of placebo or concizumab. All participants (28 healthy volunteers and 24 hemophilia patients) completed the trial, and were included in the full analysis set and safety analysis.

increase above the normal reference range of D-dimers and/or prothrombin fragment 1 + 2 ($F^1 + ^2$) for ≥ 24 h in two or more healthy volunteers and/or a maximum i.v. dose of 250 µg kg⁻¹ and a maximum s.c. dose of 1000 µg kg⁻¹ administered to healthy volunteers. Dosing between trial participants within a dose cohort was separated by ≥ 22 h to evaluate safety before dosing the next trial subject in that cohort. When all trial participants in a cohort had been dosed, a blinded safety and PK assessment was performed by an internal Novo Nordisk trial safety group prior to dose escalation. After dosing, trial participants were monitored for 43 days.

In accordance with first human dose recommendations from the European Medicines Agency [20], doses were selected based on nonclinical studies and PK/PD modeling. Dosing was initiated at the minimum anticipated biological effect level, with 0.5 µg kg⁻¹ i.v. in healthy volunteers. The s.c. dosing was started later, with 50 µg kg⁻¹ s.c. in healthy volunteers after safety had been shown with the initial i.v. doses. Subsequent dose levels were also based on PK/PD modeling.

Eligibility criteria

Eligibility criteria for healthy male volunteers and patients with severe hemophilia A or B included age 18–65 years, body weight 50–100 kg, and body mass index 18–30 kg m⁻². Healthy volunteers and hemophilia patients were ineligible if they had a low platelet count ($< 50\,000$ platelets µL⁻¹), advanced atherosclerotic dis-

ease, or history or evidence of thromboembolic events/risk (see Supporting Information for other exclusion criteria).

Concomitant treatment with FVIII and FIX concentrates

Treatment of bleeds with FVIII and FIX concentrates was allowed throughout the trial at the discretion of the investigator. Prophylaxis with regular injections of FVIII and FIX concentrates was allowed, except for 48 h before and after trial drug administration.

End points

Safety (primary) The primary end point was safety, including adverse events (AEs) over 43 days after concizumab administration. Investigators were also required to actively investigate local tolerability at injection sites. In addition, clinical laboratory assessments (e.g. hematology, biochemical parameters, coagulation-related parameters, antidrug antibodies), vital signs, physical examination, and electrocardiogram results were recorded.

Local injection-site reactions were evaluated based on the following inspections: pain or tenderness, itching, rash, redness (in mm), and induration. Coagulation-related parameters, including platelet count, protein C and S, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, and antithrombin activity were determined from citrated plasma before dosing; at 0.5, 1, 4, 8, 12, 24, 36, and 48 h after dosing; and at all other visits as safety markers. Coagulation parameters were analyzed at a central laboratory, whereas antithrombin was analyzed locally.

Evaluation of anti-concizumab antibodies in human plasma was performed centrally at the screening and dosing visits and 28 and 43 days after administration of trial product, using a bridging enzyme-linked immunosorbent assay (ELISA), with biotin-labeled concizumab for antibody capture and peroxidase-labeled concizumab for detection of bound anti-concizumab antibodies. Samples were tested in a tiered approach including screening and a confirmatory test.

Pharmacokinetics Blood samples for PK assessments were collected throughout the trial: predose and at 0 (i.e. completion of injection), 5, 15, and 30 min; 1, 4, 8, 12, 24, 36, and 48 h; and 3, 4, 5, 6, 7, 10, 14, 21, and 43 days postdosing. Blood samples from 5 and 15 min were not taken for PK assessment after s.c. administration.

Plasma concizumab concentrations were determined centrally using an ELISA, which measured free concizumab and concizumab in a 1:1 complex with TFPI.

PK endpoints included total exposure ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), clearance (CL), $t_{1/2}$, mean absorption time, volume of distribution at steady

state, bioavailability, and time to maximum concentration. Only the results of key PK parameters are presented.

Pharmacodynamics PD parameters were assessed up to 43 days postdosing at the same time points as the PK assessments. Free TFPI in plasma (i.e. TFPI not bound to concizumab) was investigated at each trial visit using an ELISA kit (Asserachrom® TFPI, Diagnostica Stago, Asnières-sur-Seine, France). Residual TFPI functionality in plasma was determined by detecting FXa activity with a chromogenic assay kit and reported as U mL⁻¹ (S2222; Chromogenix, DiaPharma Group, Inc., West Chester, OH, USA). FXa generation is inversely proportional to free TFPI concentration with increasing drug levels associated with increased FXa generation.

D-dimer and prothrombin F₁ + 2 concentrations were assessed as markers of procoagulant effect.

Statistical analysis

The sample size (four participants per cohort: three concizumab and one placebo) was chosen to enable adequate evaluation of safety and tolerability, while ensuring that the smallest possible number of trial participants were exposed to the investigational drug.

Safety analyses were based on descriptive statistics. PK parameters were determined using non-compartmental methods (via SAS version 9.3, SAS Institute, Cary, NC, USA); dose linearity for PK was evaluated based on C_{\max} and AUC_{0-∞}, and was assessed separately within i.v. and s.c. cohorts by analysis of variance on the log-transformed parameter values, using log-transformed dose as the covariate. Slope estimates were provided with 95% confidence intervals. PK parameters were estimated only for a trial subject if the plasma concentrations of concizumab recorded made it scientifically meaningful. All derived PK parameters were summarized by dose cohort. PD parameters were based on descriptive statistics, including graphs, and summarized by dose cohort and time point.

Results

Trial population

The trial was conducted from October 25, 2010, to September 10, 2012. Of 213 screened subjects, 143 healthy volunteers and 18 hemophilia patients did not meet the eligibility criteria. In total, 52 subjects (28 healthy male volunteers and 24 hemophilia patients: 21 with hemophilia A and three with hemophilia B) were randomized (Fig. 1); none had inhibitor titer > 0.6 BU. There were no withdrawals, and all participants were included in the full analysis set and safety analysis.

There were no significant differences in demographics and baseline characteristics between healthy volunteers and hemophilia patients or between s.c. and i.v. adminis-

tration. In the four cohorts (healthy volunteers i.v. and s.c. and hemophilia patients i.v. and s.c.), mean age, body weight, and body mass index ranged from 30–36 years, 71–77 kg, and 24–26 kg m⁻², respectively. None of the trial participants had clinical signs of inflammation at screening or during the study.

Safety

No serious AEs were reported during the trial (up to 43 days postdosing) in either healthy volunteers or hemophilia patients. No anti-concizumab antibodies were detected.

Across all dose cohorts, 76 AEs were reported in 34 of the 52 trial participants (Table 1). Of these, 57 (75%) were mild, 17 (22%) were moderate, and 2 (3%) were graded as severe (1 endodontic procedure in a hemophilia patient given placebo s.c., and 1 case of sciatica in a hemophilia patient given concizumab 9000 µg kg⁻¹ i.v.). However, both serious AEs were considered unlikely to be related to concizumab/placebo. All eight AEs in the highest i.v. dose cohort (9000 µg kg⁻¹) occurred in 1 patient and were of a different nature. Of the 76 AEs, 19 occurred in the placebo groups.

Across all dose cohorts, five AEs were judged by investigators as possibly or probably treatment related, of which one mild and one moderate AE occurred in the placebo group and one moderate and two mild AEs occurred after concizumab administration. The moderate AE in the concizumab group was a small superficial thrombophlebitis in a healthy volunteer in the 1000 µg kg⁻¹ s.c. cohort, manifesting as local skin tenderness 5 days post drug administration, and diagnosed with ultrasound as a short segment (1 mm thick and 1–2 cm long) of non-compressible, superficial vein corresponding with the point of tenderness. The symptoms disappeared spontaneously without treatment the day after diagnosis. The mild AEs were trace protein in urine and abdominal pain in hemophilia patients receiving 250 and 9000 µg kg⁻¹ i.v. concizumab, respectively. In the placebo group, the moderate treatment-related AE was an allergic skin reaction in a hemophilia patient on the left arm and left cheek a few hours post i.v. administration that disappeared 2 days later with treatment, and the mild treatment-related AE was injection-site discomfort in a healthy volunteer following s.c. placebo administration.

There was no apparent dose relationship with respect to the number of AEs observed. Additionally, there was no indication of a higher incidence of AEs in hemophilia patients than in healthy volunteers, except for bleeding episodes (19 AEs in patients, 0 AEs in healthy volunteers) and pain (eight AEs in patients, one AE in healthy volunteers)—both AEs that are related to hemophilia itself rather than to treatment (Table 1).

Local injection-site reactions were few: 65 mild reactions and one moderate reaction were reported in 19 trial

Table 1 Treatment-related and most common AEs following i.v. and s.c. administration of concizumab or placebo in healthy volunteers and hemophilia patients

Treatment	Total	Healthy volunteers Concizumab, $\mu\text{g kg}^{-1}$					Hemophilia patients Concizumab, $\mu\text{g kg}^{-1}$				Healthy volunteers	Hemophilia patients
		0.5	5	50	250	1000	250	1000	3000	9000	Placebo	Placebo
i.v. administration												
Trial participants (<i>n</i>)	32	3	3	3	3	–	3	3	3	3	4	4
Trial participants with AEs (<i>n</i>)	19	2	1	0	2	–	3	2	3	1	2	3
Total AEs (<i>n</i>)	37	2	1	0	3	–	5	5	3	8	2	8
Severe	1	0	0	0	0	–	0	0	0	1	0	0
Moderate	11	1	0	0	1	–	1	2	2	2	0	2
Mild	25	1	1	0	2	–	4	3	1	5	2	6
Treatment-related AEs (<i>n</i>)	3	0	0	0	0	–	1	0	0	1	0	1
							(mild)			(mild)		(moderate)
Most common AE (<i>n</i>)												
Headache, common cold, fatigue, or flu	8	2	1	0	1	–	0	1	0	2	1	0
Pain												
Musculoskeletal	3	0	0	0	0	–	0	0	1	1	0	1
Other	4	0	0	0	0	–	0	0	1	2	0	1
Bleeding												
Joint	7	0	0	0	0	–	2	1	0	1	0	3
Other	3	0	0	0	0	–	2	0	0	0	0	1
s.c. administration												
Trial participants (<i>n</i>)	20	–	–	3	3	3	–	3	3	–	3	2
Trial participants with AEs (<i>n</i>)	15	–	–	2	1	3	–	3	2	–	2	2
Total AEs (<i>n</i>)	39	–	–	5	1	6	–	13	5	–	3	6
Severe	1	–	–	0	0	0	–	0	0	–	1	0
Moderate	6	–	–	1	0	2	–	2	0	–	0	1
Mild	32	–	–	4	1	4	–	11	5	–	3	4
Treatment-related AEs (<i>n</i>)	2	–	–	0	0	1	–	0	0	–	1	0
						(moderate)					(mild)	
Most common AE (<i>n</i>)												
Skin sign or symptom	4	–	–	1	0	0	–	1	0	–	0	2
Pain												
Musculoskeletal	1	–	–	0	0	1	–	0	0	–	0	0
Other	1	–	–	0	0	0	–	1	0	–	0	0
Bleeding												
Joint	7	–	–	0	0	0	–	4	2	–	0	1
Other	2	–	–	0	0	0	–	0	1	–	0	1

AE, adverse event; i.v., intravenous; s.c., subcutaneous.

participants from a total of 3331 reported assessments in the trial. No clinically relevant changes in vital signs, electrocardiogram, or physical examination were reported following concizumab administration.

Biochemical markers including sodium, potassium, creatinine, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, γ glutamyltransferase, alkaline phosphatase, and C-reactive protein were within normal limits throughout the trial. No noteworthy change was apparent for troponin T levels, although three subjects who were dosed s.c. at 1000 or 3000 $\mu\text{g kg}^{-1}$ had marginally raised values, which were transient. Abnormal ECG recordings observed were not considered clinically significant or related to concizumab.

No clinically relevant changes in platelets, PT, aPTT, protein C and S, or antithrombin were observed. Fibrinogen levels showed high intrasubject and intersubject variation without following a trend in connection to specific

concizumab exposure levels (Fig. S3). Five healthy volunteers and three hemophilia patients had a fibrinogen value $< 1.5 \text{ g L}^{-1}$. All five healthy volunteers had baseline fibrinogen $< 2 \text{ g L}^{-1}$, and the lowest was 1.15 g L^{-1} . The percentage change was between 0% and 30%, and maximum change was often noted in the first week postinfusion. Among the hemophilia patients, two patients in the i.v. cohort receiving 1000 and 3000 $\mu\text{g kg}^{-1}$ showed a $\sim 50\%$ decrease in the first 10 days of the trial. High intra-subject and intersubject variations in the data were common, with some patients showing a significant decrease. For all observations, there were no signs or symptoms indicative of disseminated intravascular coagulation.

Pharmacokinetics

Concizumab was detected in plasma up to 43 days after dosing (Fig. 2); the plasma concentration profiles suggest

the presence of nonlinear PK, with a switch from fast clearance at low concentrations to a longer $t_{1/2}$ at higher concentrations of concizumab. The nonlinear characteristics were confirmed by PK analysis (Table 2): $AUC_{0-\infty}$ and C_{max} increased with increasing dose in a greater than dose-dependent fashion after i.v. and s.c. dosing in both healthy volunteers and hemophilia patients.

Consistent with target-mediated clearance [21], at low concizumab concentrations, binding to TFPI has a significant impact and there was faster than linear clearance; at high concizumab concentrations, a more linear clearance was observed (Table 2). Maximum mean values for $AUC_{0-\infty}$ and C_{max} were obtained in the highest dose cohorts of concizumab following i.v. administration (33 960 h $\mu\text{g mL}^{-1}$ and 247 $\mu\text{g mL}^{-1}$, respectively, for 9000 $\mu\text{g kg}^{-1}$ i.v.) and s.c. administration (2452 h $\mu\text{g mL}^{-1}$ and 16.7 $\mu\text{g mL}^{-1}$, respectively, for 3000 $\mu\text{g kg}^{-1}$ s.c.) in hemophilia patients (Table 2). In a dose-proportional context, C_{max} would be dose dependent and mean residence time (MRT), $t_{1/2}$, and CL would be dose independent, whereas for concizumab all four parameters were observed to be strongly dose related.

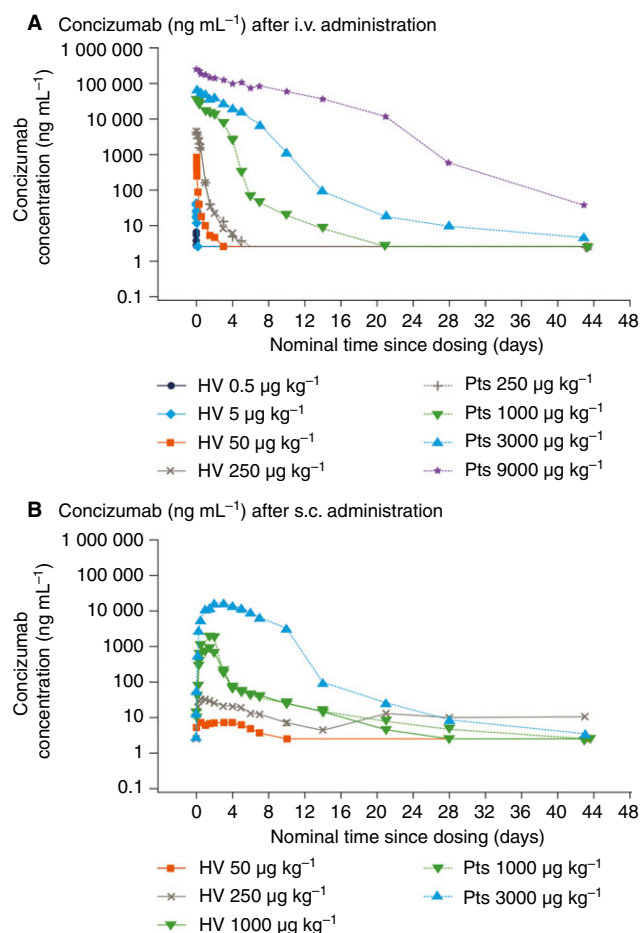


Fig. 2. Mean PK profiles of concizumab concentration (log scale) up to 43 days after i.v. and s.c. administration of concizumab or placebo to healthy volunteers and hemophilia patients.

After i.v. administration in patients, mean $t_{1/2}$ ranged from 31.1 to 74.2 h, while mean MRT ranged from 9.1 to 162 h.

The PK of concizumab was similar in healthy volunteers and hemophilia patients at the dose levels investigated in both populations (250 $\mu\text{g kg}^{-1}$ i.v. and 1000 $\mu\text{g kg}^{-1}$ s.c.). Slight differences in PK between healthy volunteers and hemophilia patients after s.c. dosing were probably due to variability of response to mode of administration rather than to differences in response between the two cohorts.

Pharmacodynamics

Free TFPI plasma concentrations decreased dose-dependently in both healthy volunteers and hemophilia patients after i.v. and s.c. concizumab administration (Fig. 3). Free plasma TFPI profiles were inversely related to PK profiles (i.e. TFPI concentration decreased with increasing concizumab concentration in plasma; Fig. 4) and remained decreased for ≥ 14 days postdosing at the highest dose levels.

Residual functional TFPI levels in plasma decreased in a concentration-dependent manner with increased concizumab exposure in both healthy volunteers and hemophilia patients. This decrease was observed at i.v. doses $\geq 250 \mu\text{g kg}^{-1}$ and at s.c. doses $\geq 1000 \mu\text{g kg}^{-1}$ (data not shown).

Coagulation activation parameters

Dose-dependent increases in D-dimer and F_{1+2} levels were observed, indicating a procoagulant effect of concizumab (Figs 5 and 6). These dose-dependent increases were generally observed at i.v. doses of concizumab $\geq 250 \mu\text{g kg}^{-1}$ in healthy volunteers, with a more pronounced effect seen in hemophilia patients only at higher i.v. doses (1000, 3000, and 9000 $\mu\text{g kg}^{-1}$). Increases in D-dimer in hemophilia patients and healthy volunteers were higher with i.v. doses than with comparable s.c. doses; this blunting to s.c. dosing may be related to the lower peaks achieved with s.c. doses. D-dimer responses in hemophilia patients were smaller than those in healthy volunteers at the same dose level; the same D-dimer concentration (ng mL^{-1}) required an ~ 36 -fold higher dose in hemophilia patients than in healthy volunteers. In contrast, F_{1+2} increases showed a tendency toward dose dependency regardless of whether they were measured in healthy volunteers or hemophilia patients (Fig. 6).

A positive correlation was demonstrated between plasma concizumab concentrations and D-dimer and F_{1+2} levels (Fig. S4A); with increasing plasma concizumab levels, the levels of D-dimer and F_{1+2} increased.

As expected, an inverse correlation was demonstrated between both D-dimer and F_{1+2} levels and free TFPI

Table 2 Mean PK parameters of concizumab following i.v. and s.c. administration in healthy volunteers and hemophilia patients

Concizumab cohort, $\mu\text{g kg}^{-1}$	C_{max} , ng mL ⁻¹ Mean (CV, %)	AUC _{0-∞} , h ng mL ⁻¹ Mean (CV, %)	MRT, h Mean (SD)	$t_{1/2}$, h Mean (SD)	CL (i.v.), mL h ⁻¹ kg ⁻¹ Mean (SD)
Healthy volunteers i.v.					
0.5	6.23 (15.3)	0.29 (11.1)	0.11 (0.03)	—	1754 (175)
5	47.9 (36.7)	36.1 (81.0)	0.73 (0.46)	—	220 (167)
50	792 (16.0)	1578 (33.6)	6.63 (3.42)	—	35 (13.4)
250	4462 (5.9)	44 932 (15.1)	8.89 (0.35)	25.7 (1.12)	5.83 (0.97)
Hemophilia patients i.v.					
250	4008 (8.7)	38 385 (9.8)	9.13 (1.06)	31.1 (3.84)	6.56 (0.73)
1000	35 525 (14.7)	1 202 469 (24.5)	35.3 (2.13)	49.5 (4.85)	0.93 (0.23)
3000	74 367 (3.1)	5 191 678 (9.3)	68.9 (2.21)	74.2 (16.1)	0.57 (0.07)
9000	247 104 (30.8)	33 960 277 (30.0)	162 (20.4)	65.9 (11)	0.28 (0.08)
Healthy volunteers s.c.					
50	9.16 (27.3)	—	—	—	—
250	35.9 (18.8)	10 772 (93.4)	274 (246)	90.3 (18.2)	37.5 (22.7)
1000	999 (42.7)	54 695 (36.8)	85.9 (22.3)	114 (5.46)	19.9 (6.71)
Hemophilia patients s.c.					
1000	1791 (88.6)	109 350 (70.8)	126 (137)	116 (69.4)	17.2 (17.9)
3000	16 689 (42.4)	2 452 323 (52.0)	105 (11)	74.8 (31.4)	1.63 (1.17)

CL, clearance; CV, coefficient of variation; i.v., intravenous; PK, pharmacokinetics; MRT, mean residence time; s.c., subcutaneous; SD, standard deviation; $t_{1/2}$, terminal half-life.

plasma concentrations (Fig. S4B). The highest increases of D-dimers and F_{1+2} were observed when TFPI could no longer be detected by the TFPI ELISA, suggesting potential saturation of plasma TFPI pool and inhibition of TFPI associated with microvasculature.

Bleeding events

Twenty-four bleeds/bruises occurred in 14 hemophilia patients (nine concizumab; five placebo), and one bruise was reported in a healthy volunteer given concizumab (Table S1). None of the bleeds occurred at high concizumab levels and low free TFPI plasma concentrations, except a bleed caused by a minor finger cut (Fig. 4).

Discussion

This trial was the first to assess the safety, PK, and PD of single-dose concizumab in humans. Importantly, there were no safety concerns with concizumab after i.v. or s.c. administration. There were no serious or severe treatment-related AEs, and no antidrug antibodies were detected. Local injection-site reactions were few; one moderate reaction, a superficial thrombophlebitis in a healthy volunteer in the 1000 $\mu\text{g kg}^{-1}$ s.c. cohort, was manifest as local skin tenderness 5 days postdrug administration, which quickly disappeared spontaneously.

Nonlinear PK of concizumab was observed after i.v. and s.c. dosing in healthy volunteers and patients, reflecting dose-dependent, target-mediated clearance at low doses. Target-mediated clearance is commonly observed with monoclonal antibodies and indicates that binding of

the drug (monoclonal antibody) to its target influences drug distribution and/or elimination and consequently the drug concentration. Thus, low concizumab concentrations were associated with faster clearance due to binding to TFPI, and high concizumab concentrations were associated with a slower clearance typical of monoclonal antibodies since clearance via the target contributes less to the overall clearance at high drug concentrations.

Free TFPI plasma concentrations and residual TFPI functional levels decreased in a concizumab concentration-dependent manner in healthy volunteers and hemophilia patients after i.v. and s.c. administration.

Post concizumab administration, there were no clinically relevant changes in platelets, PT, aPTT, and anti-thrombin. There was a decrease in mean fibrinogen concentration, which continued to be within normal range. Furthermore, interindividual variability in fibrinogen levels was common, with some patients in the highest dose cohort showing a significant decrease. As expected, there was a dose-dependent procoagulant effect of concizumab, seen as increased levels of D-dimers and prothrombin F_{1+2} ; although indirect, this evidence confirms the procoagulant activity of concizumab.

At similar dose levels the procoagulant response was greater in healthy volunteers than hemophilia patients, and the magnitude of response appeared to be more pronounced with i.v. than s.c. administration. The latter is potentially due to slower absorption of the antibody, with lower peak levels and lesser inhibition of TFPI. Further peak responses were beyond the measurable range of the TFPI assay—consistent with the known distribution of the TFPI, where more than 50–80% is associated with endothelium [22].

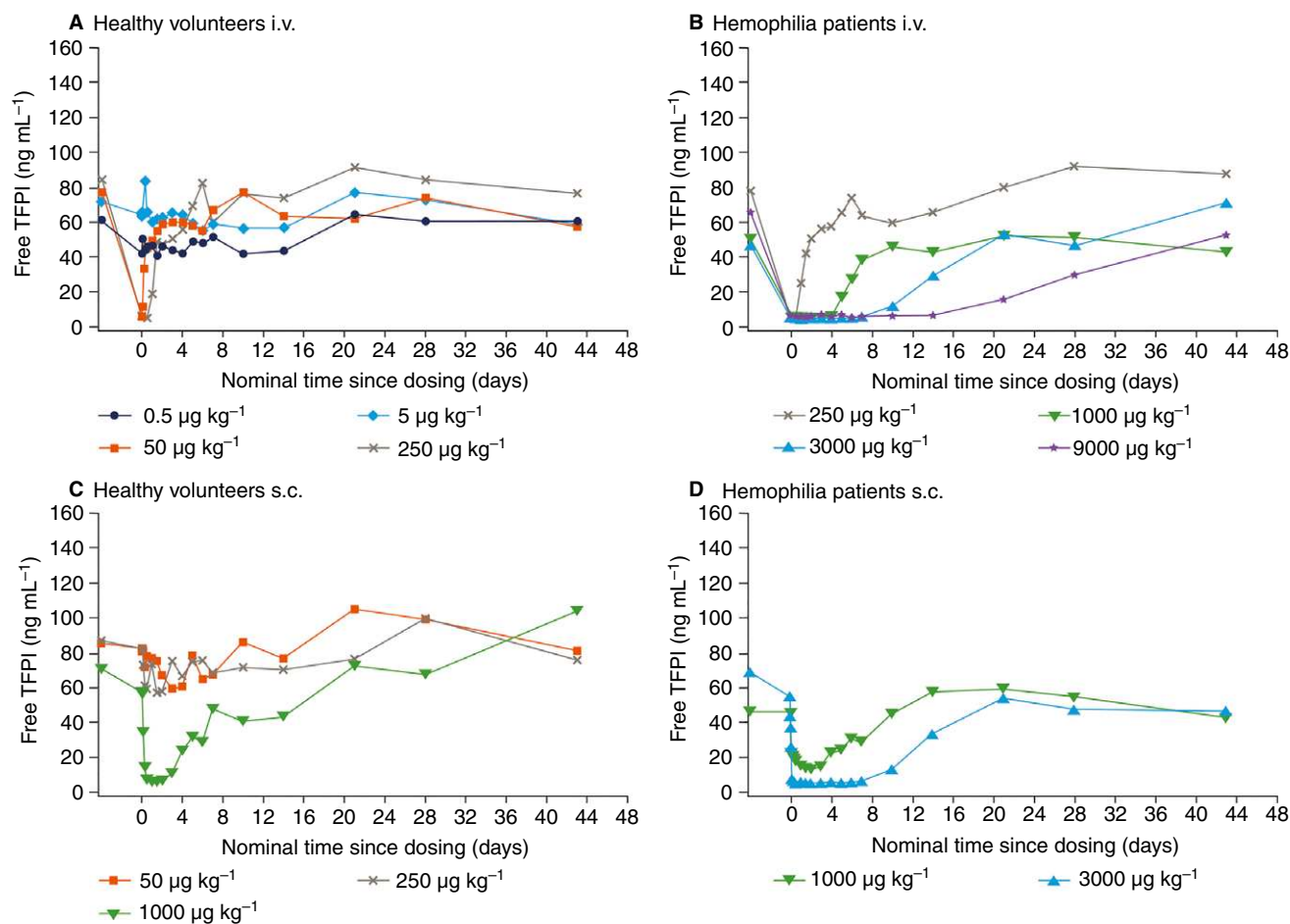


Fig. 3. Mean free TFPI plasma concentration (ng mL⁻¹) evaluated by TFPI ELISA up to 43 days after i.v. and s.c. administration of concizumab or placebo to healthy volunteers and hemophilia patients.

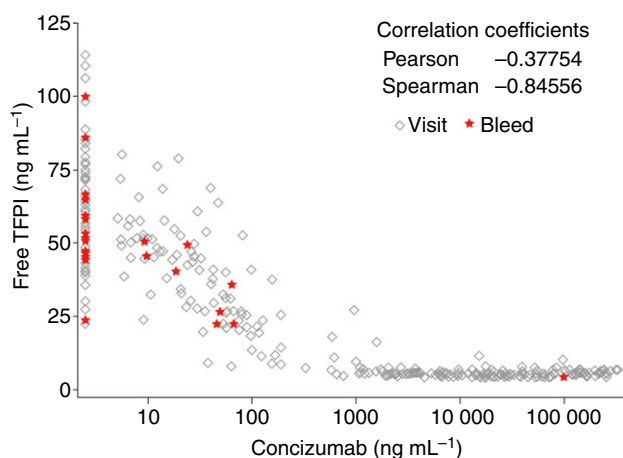


Fig. 4. Scatterplot of free TFPI vs. concizumab levels. Patients had also been treated with FVIII; the bleeding experienced by a patient when concizumab was at a high level was a minor finger cut.

The PK profile observed in rabbits similarly revealed a nonlinear, dose-dependent profile, consistent with a target-mediated clearance [21]. Further, rabbit and rat

models investigating the biodistribution of concizumab by immunohistology showed marked co-localization of concizumab with endogenous rabbit TFPI on the endothelium of the microvasculature with negligible subendothelial build-up [23].

In a monkey model the maximum target clearance was estimated at $11 \mu\text{g h}^{-1} \text{kg}^{-1}$ and the K_m (the concentration at which the target-mediated elimination rate is 50% of the maximum) for concizumab was $0.54 \mu\text{g mL}^{-1}$ where the nonlinear part of the clearance predominates [21]. The data suggest that TFPI, the target for concizumab, constitutes a pool with a considerable capacity for clearance of the administered antibody.

Regarding bleeding events, none occurred at high concizumab levels or low free TFPI plasma concentrations, except a minor finger cut. Although bleeds are expected as a part of the patients' underlying hemophilia, bleeds were reported as AEs. This trial was not aimed at evaluating hemostatic efficacy on bleeding rate, and patients were allowed to treat themselves with their standard FVIII or FIX concentrate both on demand and prophy-

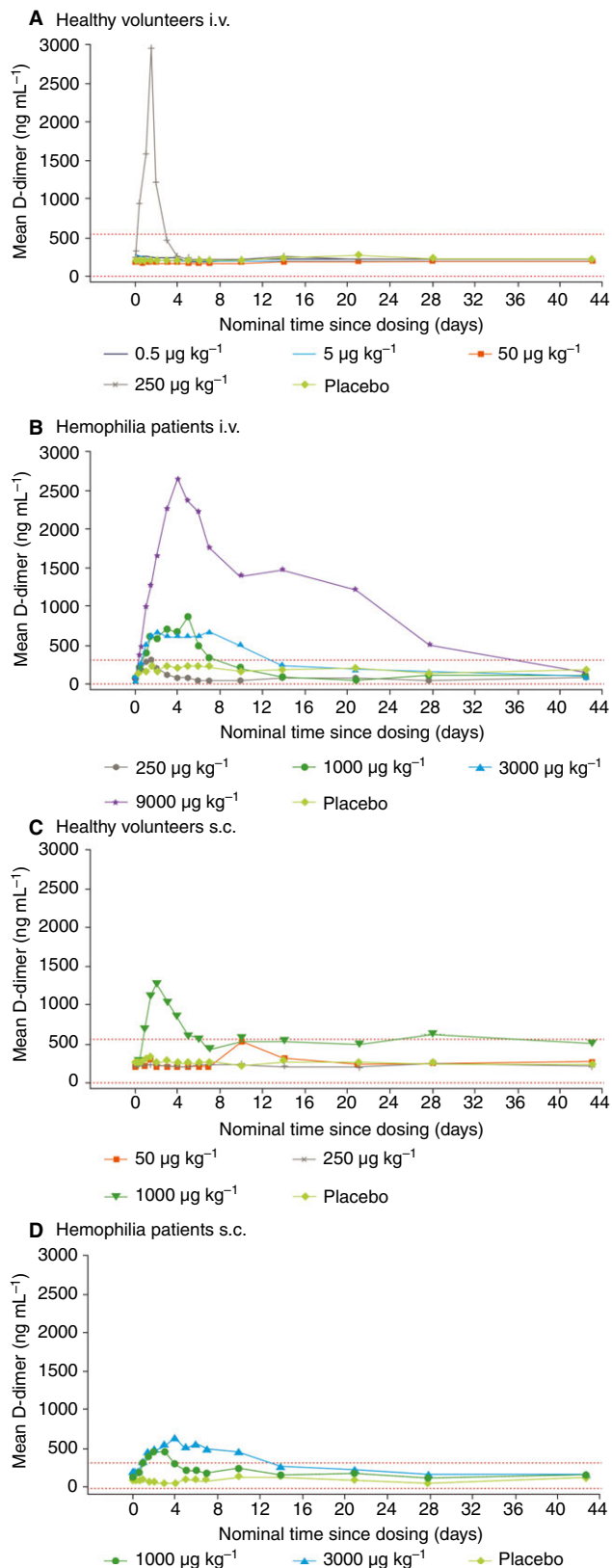


Fig. 5. Dose-dependent changes in mean D-dimer levels up to 43 days after i.v. and s.c. administration of concizumab or placebo to healthy volunteers and hemophilia patients. The horizontal red dashed line represents the reference ranges for D-dimers (ng mL⁻¹).

lactically. Therefore, the bleeding data must be interpreted with caution.

Monoclonal antibodies have several potential advantages in the treatment of patients with hemophilia, including s.c. administration. Importantly, antibody therapy is not associated with the development of inhibitors against coagulation factors. Other advantages of antibody drugs include good solubility and stability, a long plasma $t_{1/2}$, high selectivity, and specificity [24].

Due to its mode of action, concizumab has the potential to prevent bleeding in both hemophilia A and B, irrespective of inhibitor status or severity. For the first time, venous access is not required with this hemophilia treatment, with potential for improved patient compliance and, therefore, outcomes.

These data should be viewed in light of the limited size of the cohorts and the single-dose exposure. However, this first-in-human clinical trial generated valuable PK data, and will provide the basis for future safety and efficacy trials. Clinical trials are required to confirm the hemostatic efficacy of concizumab in patients with hemophilia and to define the therapeutic window of TFPI inhibition. The dose-finding studies will need to address the impact of target-mediated clearance on frequency of administration and dosing of concizumab, and its clinical application regarding prophylaxis and bleeding.

In conclusion, concizumab has the potential to alter current concepts of prophylaxis in patients with hemophilia. There were no safety concerns with concizumab after i.v. or s.c. administration in healthy volunteers or in patients with hemophilia. PK investigations showed that concizumab was influenced by target-mediated clearance. A concentration-dependent effect was observed on plasma TFPI functionality and levels. The results of ExplorerTM1 are promising, and the potential use of s.c. concizumab for hemophilia treatment warrants further study.

Addendum

P. Chowdary performed the research, analyzed the data, contributed to writing the manuscript, and reviewed and approved the manuscript. S. Lethagen and U. Friedrich were involved in designing and monitoring the trial and in analyzing and interpreting the data, drafted and contributed to writing the manuscript and revising it critically for important intellectual content, gave final approval, and agreed to be accountable for the work. All other authors performed the research, reviewed the data, and critically reviewed and approved the manuscript. P. Angchaisuksiri analyzed the data.

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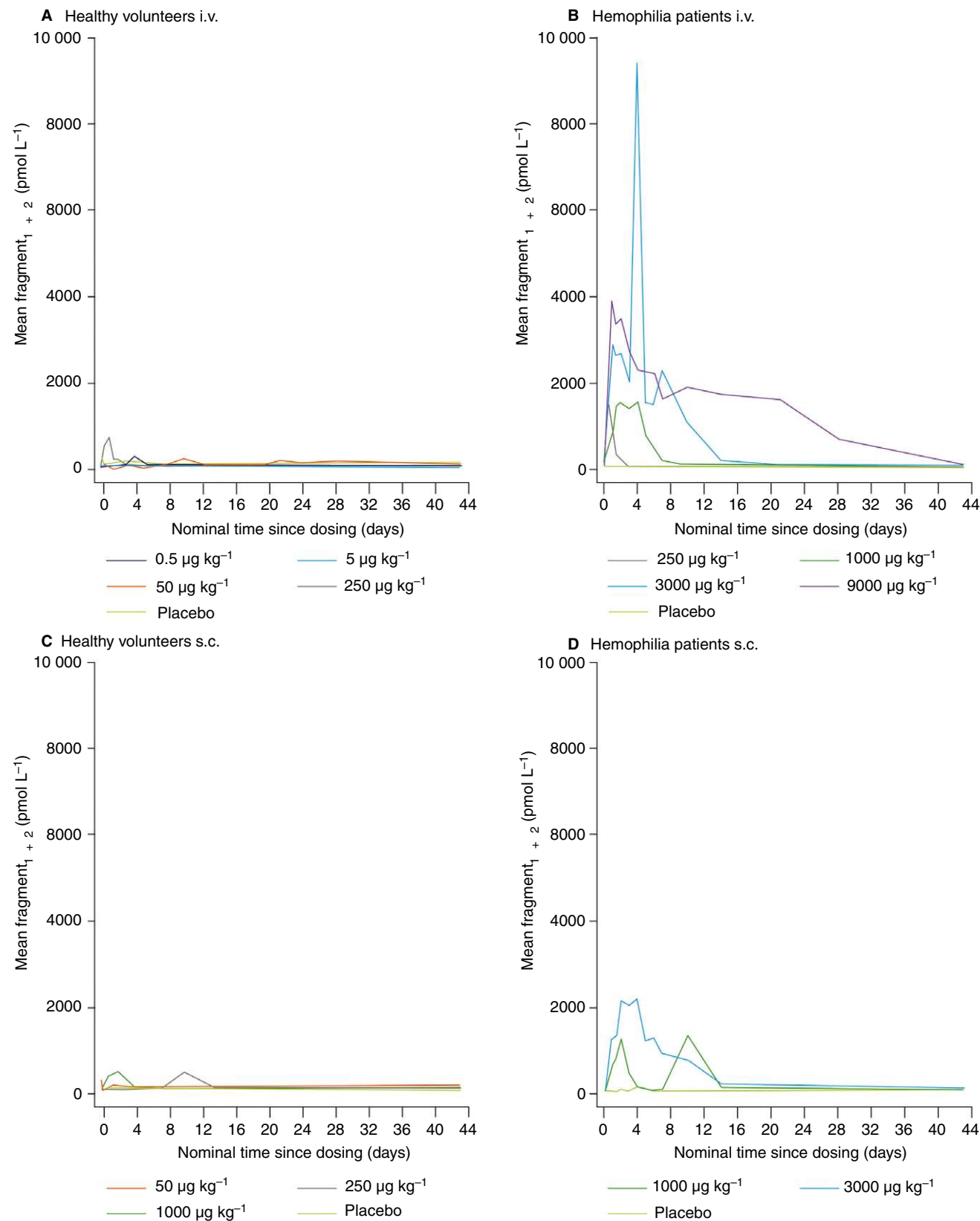


Fig. 6. Dose-dependent changes in mean F_{1+2} levels up to 43 days after i.v. and s.c. administration of concizumab or placebo to healthy volunteers and hemophilia patients.

site was approved by the appropriate ethics committees or institutional review boards but did not recruit any patients. The authors also thank M. B. Petersen and A. Rosholm from Novo Nordisk A/S for their work on the PK and statistical analyses for this study and manuscript.

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Disclosure of Conflict of Interests

P. Chowdary has received grants, personal fees, and non-financial support from Novo Nordisk, during the conduct of the study; grants, personal fees, and non-financial support from Pfizer and CSL Behring; personal fees and non-financial support from Bayer, Baxter, and Biogen Idec; and non-financial support from Grifols, outside the submitted work. S. Lethagen was a full-time employee of Novo Nordisk during this study and a shareholder of Novo Nordisk shares. Since October 2013, he has been a full-time employee of Sobi and a shareholder of Sobi shares. U. Friedrich is a full-time employee of Novo Nordisk. B. Brand has received personal fees from Novo Nordisk AG, Bayer AG, Baxter AG, CSL Behring AG, Pfizer AG, and Biotest AG, outside the submitted work. R. Klamroth has received grants from Novo Nordisk, during the conduct of the study; and grants and personal fees from Novo Nordisk, Bayer, Baxter, CSL Behring, Pfizer, SOBI, Biogen Idec, and Octapharma, outside the submitted work. P. Knoebel has received personal fees and other support from Novo Nordisk, during the conduct of this study; grants, personal fees, and other support from Baxter, and other support from CSL Behring and Boehringer, outside the submitted work. M. Laffan has received grants and other support from Novo Nordisk, during the conduct of this trial, and personal fees from Roche, Bayer, Baxter, and Pfizer, outside the submitted work. J. Mahlangu received research grants from Novo Nordisk and personal fees and research grants from Bayer, Biogen, and CSL Behring. C. Hay, F. Abdul Karim, W. Miesbach, J. Dalsgaard Nielsen, M. Martín-Salces, and P. Angchaisuksiri have no conflicts of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Concizumab and tissue factor pathway inhibitor (TFPI) mode of action.

Fig. S2. Schematic structure of TFPI α .

Fig. S3. Fibrinogen concentrations up to 43 days after i.v. and s.c. administration of concizumab or placebo to hemophilia patients.

Fig. S4. D-dimer and F₁ + 2 levels at (A) increasing concentrations of concizumab and (B) increasing free TFPI plasma concentrations in healthy volunteers and hemophilia patients (data are pooled across i.v. and s.c.).

Table S1. Timing of bleeding episodes according to i.v. and s.c. dose cohort (concizumab or placebo) in a healthy volunteer and hemophilia patients.

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